Please add the following claim:

--78. The recombinant virus vector of claim 52, wherein the regulatory nucleic acid sequence comprises a sequence of approximately 800 nucleic acid residues located upstream of the transcription start point of the myosin light chain 2 gene.

CH

79. The recombinant virus vector of claim 52 that is effective to obtain heart- or heart cavity-specific expression of said nucleic acid to be expressed and does not comprise a CSS regulatory element consisting of nucleotides 682 to 724 of SEQ ID NO: 1.--

REMARKS

Applicants filed a Supplemental Amendment on May 18, 2000. However, it appears that the Examiner did not consider the Supplemental Amendment. Courtesy copies of the Supplemental Amendment and the mailroom postcard receipt are attached hereto for the Examiner's convenience. The Examiner is respectfully requested to enter and make the Supplemental Amendment of record in the present application.

The Office Action of June 28, 2000 presents the examination of claims 20-73. Claims 20-51, 74, and 75 are canceled. Claims 52-53, 58-59, and 69 are amended. Support for the amendment of claim

52 is found on page 2, paragraph 3, to page 3, paragraph 1 of the specification. Support for the amendment of claim 69 is found on page 11, and Example 8 of the specification. Claims 78 and 79 are added for consideration of the Examiner. Support for claim 78 is found on page 4, last paragraph, of the specification. Support for claim 79 is found in the original claim 21. No new matter is inserted into the application.

Rejection under 35 U.S.C. § 101

The Examiner rejects claims 45-51 under 35 U.S.C. § 101, for allegedly being directed to non-statutory subject matter. Claims 45-51 are canceled, thus rendering the instant rejection moot. However, the Examiner is advised that a regulatory nucleic acid sequence is a molecule and therefore is statutory subject matter.

Rejection under 35 U.S.C. § 112, second paragraph

The Examiner rejects claims 20-51 and 53 under 35 U.S.C. § 112, second paragraph for allegedly being indefinite. Claims 20-51 are canceled, thus rendered the rejection applied to said claims moot. Applicants respectfully traverse the rejection of claim 53. Reconsideration of the claim and withdrawal of the instant rejection are requested.

Specifically, the Examiner asserts that the phrase "functional homolog" is indefinite. In response to the Examiner's remarks, Applicants delete said phrase from claim 53.

Applicants respectfully submit that the amendment of claim 53 renders the claim in full compliance with 35 U.S.C. § 112. Thus, the instant rejection is overcome.

Rejection under 35 U.S.C. § 112, first paragraph

The Examiner rejects claims 40-44 and 69-73 under 35 U.S.C. § 112, first paragraph, for allegedly not being described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. Claims 40-44 are canceled, thus rendering the rejection applied to said claims moot. Applicants respectfully traverse the rejection of claims 69-73. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

The Examiner rejects any claims directed to a pharmaceutical composition. Applicants amended claims 72 and 73 to simply recite a composition, not a pharmaceutical composition, in the Supplemental Amendment of May 18, 2000 following an Interview with the Examiner. In the Interview, it was discussed that the amendment of the claims to recite a "composition" rather than a "pharmaceutical composition" would be remedial. As no claims

pending in the present application recite a "pharmaceutical composition", this ground of rejection is overcome.

On page 4 of the Office Action, the Examiner notes that the Examples provided in the specification teach that the vectors of the present invention provide an effective gene delivery system via injection to the desired cells. However, the Examiner goes on to state that this does not enable delivery of the vector constructs by other routes. Claims 69-71 are directed to a method for delivery of a desired gene to cardiac muscle cells. Applicants amend claim 69 (and therefore dependent claims 70 and 71) to recite that delivery of the desired gene is done intravenously or by injection into the cardiac cavity or pericardial space, as disclosed on page 11, and Example 8 of the specification.

The present invention provides an effective delivery system for gene therapeutic applications. The specification provides data demonstrating efficiency when the desired gene is delivered intravenously or by injection into the cardiac cavity or pericardial space. As the present claims recite such a route of delivery, the instant rejection is overcome.

Rejection under 35 U.S.C. § 102(b)

The Examiner rejects claims 20, 22-28, 33, 38, and 45-51 under 35 U.S.C. § 102(b) for allegedly being anticipated by Franz,

Arnold, Knowleton, Shubeita, Navankasattusas, Thornburn, or Goswami. Claims 20, 22-28, 33, 38, and 45-51 are canceled, thus rendering the rejection applied to said claims moot.

Rejection under 35 U.S.C. § 103(a)

The Examiner rejects claims 20-39 and 45-68 under 35 U.S.C. § 103(a) as being unpatentable over Franz, Arnold, Knowleton, Shubeita, Navankasattusas, Thornburn, and Goswami in view of Ricigliano and Zaia. Claims 20-39 and 45-51 are canceled, thus rendering the rejection applied to said claims moot. Applicants respectfully traverse the rejection of claims 52-68. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

The Present Invention

The present invention provides a vector construct that allows for cardiac specific expression of a gene of interest in an animal body under *in vivo* conditions, particularly under conditions of somatic gene transfer.

The present inventors were the first to show that a gene of interest, under the control of a myosin light chain 2 gene promoter, could be specifically expressed in cardiac tissue under

in vivo conditions of somatic gene therapy. This finding was completely unexpected for several reasons, as explained below.

None of the cited prior art references disclose or suggest the use of the myosin light chain 2 promoter with a viral construct. Those skilled in the art at this time were aware of the non-specificity of a virus and thus would have avoided the use of such viral constructs for cardiac specific gene transfer. Thus, the skilled artisan would have no reasonable expectation of success if he relied upon the disclosures of the cited prior art references.

Second, the results observed from germ line manipulation do not provide a basis for predicting the behavior of the regulatory sequence under conditions of somatic gene therapy. In comparative experiments published in 1997 in *Cardiovascular Research* (Franz et al. *Cardiovascular Research* 35:560 (1997), copy attached), Franz and co-workers observed that tissue specific gene expression of the 1.0 kb α MHC (myosin heavy chain) promoter and the 0.8 kb myosin light chain 2 promoter in an adenoviral vector system are completely different. At this time, it was known in the art that both promoter sequences directed cardiac-specific protein expression *in vivo* in a transgenic animal model after germ-line manipulation. However, it was not known whether cardiac specificity would be conserved when the promoters were used in an adenoviral vector system. Several injection experiments were

therefore performed with neonatal rats under conditions of somatic gene therapy. The results showed that while the 0.8 kb myosin light chain 2 promoter of the present invention maintained specificity when used in a adenoviral system, the prior art 1.0 kb α MHC promoter lost cardiac specificity. In fact, the gene under control of the α MHC promoter was expressed in the lung and liver in addition to a cardiac tissue.

Thus, these results demonstrate that the behavior of a promoter when used in somatic gene therapy versus germ-line therapy cannot be predicted from results obtained from experiments obtained from germ-line manipulations. These observed differences are even more significant when it is taken into account that the two promoter sequences (α HMC and MLC-2) are functionally related and direct expression of different chains of the same protein (myosin), which is a major component of heart muscle and other striped muscles.

Furthermore, the results of Example 11 of the specification illustrate that adenoviral constructs containing the α MHC promoter or the MLC-2 promoter differ not only with regard to specificity, as described above, but also in their activity. The present inventors unexpectedly observed that the MLC-2 promoter induces a 3 to 4 times stronger cardiac specific expression than the α MHC

promoter under conditions of somatic gene therapy. Further, the results of Example 12 show that only the MLC-2 promoter of the present invention allows for ventricle-specific gene expression. Thus, the present invention provides a specific tool for targeting trans-gene expression to the ventricular heart muscle under conditions of somatic gene therapy, that is not provided by or suggested by the prior art references.

Distinctions between the Present Invention and the Combination of the Prior Art References

None of the prior art references disclose or suggest the use of an expression construct comprising a myosin light chain 2 promoter for therapeutic applications. The Examiner attempts to make up for the deficiencies of Franz, Arnold, Knowleton, Shubeita, Navankasattusas, Thornburn, and Goswami by combining these disclosures with Ricigliano and Zaia. The Examiner writes on page 11 of the Office Action of September 21, 1999 that,

> One of ordinary skill in the art would have a reasonable expectation of success for cardiac specific expression of an antisense molecule from the MLC-2 promoter because Ricigliano et al. broadly teaches use of tissue-specific promoters, like MLC-2, to heterologously express antisense, and functionally equivalent ribozymes.

Applicants respectfully disagree. In fact, neither disclosure of Ricigliano and Zaia suggests a reasonable expectation of success for cardiac specific expression; (1) neither reference discloses cardiac specific expression and (2) neither reference provides evidence that would support their assertions of tissue-specific expression.

For example, Ricigliano does not even refer to the specific technical field of cardiac specific gene transfer under conditions of somatic gene therapy. The alleged usefulness (see col. 4, II. 52) "for ameliorating the effects of diseases of muscle by expression of the normal gene..." has not been supported by a single piece of experimental evidence. The muscle specific promoter used by Ricigliano (i.e. MCK) is expressed in both skeletal and cardiac muscle (see col. 3, lines 32-33). Thus, the MCK promoter should not be regarded as providing cardiac tissue specific expression as recited in the present claims. Moreover, Ricigliano has not at all investigated efficacy of his promoter in a viral vector system. Ricigliano also has not at all provided experimental evidence that, under the control of the MCK promoter, muscle specificity of expression is maintained.

Zaia also fails to support any allegations of tissue-specific expression. Specifically, no *in vivo* experiments are disclosed. Furthermore, the authors admit: "The in vivo efficacy of

conferring intracellular resistance to HIV-1 remains unproven" (see p. 102, last paragraph). In addition, Zaia fails along with Ricigliano to refer to the specific technical field of cardiac specific gene transfer under conditions of somatic gene therapy.

For these reasons, there is no basis for Examiner's assumption that one skilled in the art would have considered the teaching of the prior art documents as establishing a reasonable chance of success. The combination of prior art documents simply fails to anticipate or predict the unexpected results provided by the present invention.

Applicants submit that the present application describes and claims patentable subject matter, and therefore respectfully request that the Examiner withdrawal the rejection made under 35 U.S.C. § 103. Prompt, favorable action of allowance of the claims is respectfully requested. If there are any minor matters precluding allowance of the application which may be resolved by a telephone discussion, the Examiner is respectfully requested to contact Kristi L. Rupert, Ph.D. (Reg. No. 45,702) at (703) 205-8000.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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GMM/DRN/KLR
0690-0110P

Attachments: Copy of Previous Supplemental Amendment
Franz et al.